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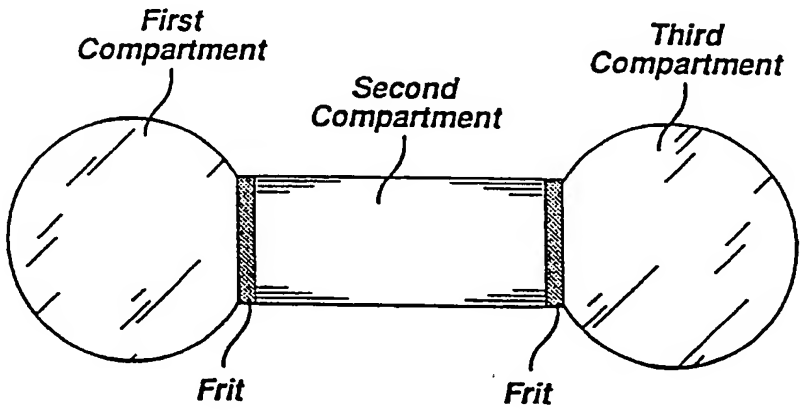

(57) Abstract

There is disclosed and improved process for detecting a particular motile organism in a competitor immobilization test, wherein competitor flagella antibodies are added to either or both of the selective enrichment medium and/or a semisolid motility medium for the purpose of restricting the movement of motile competitors within either or both media. The addition of competitor flagella antibodies results in greater sensitivity of the competitor immobilization procedure and fewer false-negative results than had been achieved with motility procedures.

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Description

"PROCESS AND DEVICE FOR DETECTING A PARTICULAR MOTILE ORGANISM"

Technical Field of the Invention

The present invention relates to an improved process for detecting a particular motile organism within a sample. More particularly, the present invention relates to the use of a plurality of antibodies specific to epitopes on flagella of motile competitors to prevent movement of the motile competitors without inhibiting movement of the particular organism for the purpose of enhancing detection of the particular motile organism.

Terminology And Background of the Invention

The terminology used herein will be "particular motile organism" to describe the specific microorganism or bacterial pathogen that the inventive process is designed to detect. "Motile competitors" refers to other motile microorganisms (except the particular motile organism) that may be in a sample. "Motile organisms" refers to the combination of the motile competitors and the particular motile organism, or the combination of all motile organisms within a sample. An "enriched sample" refers to a test sample that had been added to a culture medium and incubated at a permissive temperature for a certain period of time to permit growth of the particular motile organism.

The terminology used herein includes two types of antibodies. The particular antibodies are specific for epitopes on the flagella of the particular motile organism. The competitor flagella antibodies are specific for epitopes on the motile competitors. The competitor flagella antibodies do not substantially cross-react with sites on the particular motile

organisms, and likewise, the particular antibodies do not substantially cross-react with motile competitors.

It should be noted that when, for example, Salmonella is the particular motile organism, then Escherichia coli and Yersinia entercolitica may be the motile competitors. Similarly, when E. coli is the particular motile organism, Salmonella may be considered a motile competitor. Thus, an organism may be either a particular motile organism or a motile competitor, depending upon the test being performed.

The competitor flagella antibodies act to confer selectivity in a motility or an enrichment test procedure.

The detection of bacteria in food, water, waste water, environmental, pharmaceutical, cosmetic and clinical samples is often complicated by the fact that the microorganism (particular motile organism) may be present in the sample at concentrations much lower than those of other microorganisms (i.e., motile and nonmotile competitors).

Competitor microorganisms, including motile competitors, can often interfere with the detection of a pathogen, or a particular motile organism, by overgrowing on the surface of an agar plate or by inhibiting the growth of the particular motile organism in a culture broth. An isolated colony of the particular motile organism may not appear on the surface of an agar plate if large numbers of colonies of competitor organisms are present. The competitor organisms may consume essential nutrients and oxygen, release by-products that possess antibacterial properties, or raise or lower pH to levels which inhibit bacterial growth or kill the organism. As a consequence, the competitor organisms may grow to extremely high concentrations in the culture broth, whereas the bacterial pathogen (i.e., particular motile organism) remains below detectable limits or can actually die off.

For example, Salmonella is a genus of facultative, anaerobic, Gram-negative, flagellate bacteria which may be found in a variety of food stuffs, dairy products, feed, water and waste water, and which are a cause of various pathological conditions in humans. Salmonellosis is a food-associated disease and a major problem in the United States. However, salmonellosis is usually not a fatal disease, although some fatalities have been reported in infants, immunocompromised individuals and the aged.

Food, dairy and feed industries routinely scrutinize their products for Salmonella contamination. Assays for Salmonella contamination in products is complicated by the fact that the Salmonella organism is commonly present at extremely low levels. The presence of numerous other microorganisms (i.e., competitor organisms), including competitive flagellate species (motile competitors), can further complicate the detection of Salmonella. Competitor organisms, including motile competitors, can interfere with the detection of Salmonella by pure culture techniques.

The pure culture technique used for conventional detection of, for example, Salmonella, is cumbersome and labor-intensive to perform, requires expensive laboratory materials, requires at least four days to complete the procedure, and may yield false-negative results. The pure culture technique for the detection of Salmonella from foods involves multiple enrichment and plating steps which are intended to minimize the problems associated with the presence of competitor organisms.

The first step in the pure culture detection sequence for Salmonella involves the introduction of the sample into a preenrichment broth and incubation for 24 hours at 35°C. The purpose of this first step is to promote the repair of the Salmonella (i.e., particular motile organism) if it has been damaged due to process-

ing of the food, and to encourage the growth of the particular motile organism. This first pre-enrichment step, however, also results in the growth of the competitor organisms, including motile competitors. If the competitor organisms are present in the food at high levels, or if the competitor organisms are capable of growing more rapidly than the particular motile organism, then the competitor organisms may reach levels in the pre-enrichment broth, after incubation, which are 1,000 to 10,000,000 times greater than the particular motile organism.

The next step in the detection sequence for the pure culture method is the transfer of a small volume of the pre-enrichment broth into selective enrichment broths. The selective broths include certain chemical agents that suppress the growth of the competitor organisms while permitting the growth of the particular motile organism. The selective enrichment broths are incubated at 35°C or 43°C for approximately 24 hours. The higher incubation temperature is sometimes used to further suppress the growth of the competitor organisms.

Three types of agar plates, each containing different selective agents to inhibit the growth of competitor organisms, are usually streaked from the selective broths after incubation of the selective enrichment broths. These plates are incubated for 24 hours at 35°C, and then examined for the presence of colonies of Salmonella.

A commercial screening test is available for the rapid and simple detection of motile Salmonella in a sample. This test, the 1-2 TEST® from BioControl Systems, Inc., Bothell, Washington, is described in United States Patent No. 4,563,418, the disclosure of which is incorporated by reference herein. This test utilizes a particular organism immuno-immobilization procedure. The 1-2 TEST® uses an immunological

detection system to immobilize the particular motile organisms. The 1-2 TEST® is packaged in a disposable plastic container with two interconnecting chambers. One chamber contains tetrathionate broth, brilliant green dye and serine, which is a selective enrichment broth for Salmonella. The other chamber contains a semisolid motility medium. An enriched sample is inoculated into the first chamber, containing the selective enrichment broth. A drop of a particular antibody preparation, which reacts specifically with the flagella of Salmonella, is added onto a distal surface of the semisolid motility medium in the second chamber. The inoculated unit is incubated at 35°C. The motile Salmonella and certain motile competitors move from the selective enrichment broth into the semisolid motility medium. The particular motile organisms progress through the semisolid motility medium, eventually interacting with the particular antibodies that have diffused radially into the semisolid motility medium. The particular motile organisms become incapable of further progression through the semisolid motility medium upon reaction of the flagella of the particular motile organism with the diffusing particular antibodies. The immobilized cells form a whitish band that can be visually read. Any motile competitors that progress with the particular motile organisms through the semisolid motility medium do not interact with the diffusing particular antibodies and continue to move to the distal end of the semisolid motility medium.

Specificity of the 1-2 TEST® system and similar tests is partially conferred by the selective enrichment medium, allowing preferential growth of the particular motile organism relative to the motile competitors. The use of selective agents in the selective enrichment medium is a means for conferring selectivity for the particular motile organism. Selective agents for Salmonella include, for example, brilliant

green dye, bile salts, sodium dodecyl sulfate, novobiocin, sodium selenite and other agents. These agents, however, can act to inhibit the growth of certain sensitive strains of the particular motile organism and prevent it from growing to detectable limits. For example, Schothorst et al., "Studies on the Multiplication of Salmonellae in Various Enrichment Media at Different Incubation Temperatures," J. Appl. Bact. 42:157-63, 1977, found several Salmonella strains that did not grow in the presence of brilliant green dye. Further, Greenfield et al., "Selective Inhibition of Certain Enteric Bacteria by Selenite Media Incubated at 35°C and 43°C," Can. J. Microbiol. 16:1267-71, 1970, found that strains of the Salmonella serotypes S. choleraesuis, S. gallinarum, S. pullorum and S. typhi did not grow well with sodium selenite. Further still, Vassiliadis et al., "Evaluation of the Growth of Salmonellae in Rappaport's Broth and in Muller-Kauffmann's Tetrathionate Broth," J. Appl. Bact. 37:411-18, 1974, examined 70 strains of Salmonella and found several strains that did not grow in the selective broth tested. Thus, the selective agents may also inhibit the growth of certain Salmonella serotypes. Therefore, there is a need in the art for a better means for conferring selectivity for the particular motile organism in a microbiological test.

The presence of large numbers of motile competitors in the tetrathionate broth (i.e., selective enrichment medium) in the first chamber of a Salmonella test can interfere with the movement of Salmonella into the semisolid motility medium. In general, the presence of large numbers of motile competitors can decrease the sensitivity of any microbiological test. This can result in false-negative readings. Presently, this problem can be partially solved by using a selective enrichment step before inoculation. The drawback to the use of a selective enrichment step prior to the

beginning of the microbiological test is that this procedure lengthens the test time by about 24 hours. Therefore, there is a need in the art for a means to reduce false-negative results in a microbiological test when there are large numbers of motile competitors present in a sample, without significantly increasing test time.

Summary of the Invention

The present invention is directed to the use of competitor flagella antibodies that allow the preferential detection of a particular motile organism, through a competitor immobilization procedure. More specifically, the present invention reduces the number of false-negative test results encountered in a particular organism immobilization procedure in the presence of large numbers of motile competitors. The inventive method also improves microbiological procedures by modifying the semisolid motility medium and, in certain aspects, the selective enrichment medium. Such a modification includes the addition of a plurality of competitor flagella antibodies to the semisolid motility medium alone, the selective enrichment medium alone, or to both the semisolid motility medium and the selective enrichment medium. Preferably, the competitor flagella antibodies are added only to the semisolid motility medium.

The plurality of competitor flagella antibodies function to immobilize the motile competitors within either or both media. In this way, the competitor flagella antibodies act as a means for conferring selectivity for the particular motile organism. The competitor flagella antibodies react specifically with the flagella of motile competitor bacteria, but do not significantly react with the flagella of the particular motile organisms.

The competitor flagella antibodies are incorporated into the semisolid motility medium and/or selective enrichment medium contained in the detection unit of a competitor immobilization test. The competitor flagella antibodies prevent or retard the movement of motile competitors within the media of the detection unit without appreciably retarding the movement of the particular motile organism. This permits the particular motile organism to move exclusively or in predominant numbers into and through the semisolid motility medium for detection. One means for detecting the particular motile organism is to have diffusing antibodies react specifically with the particular motile organism. The incorporation of the competitor flagella antibodies therefore increases the selectivity of the assay and permits detection of low levels of the particular motile organism in the presence of high levels of motile competitors without a high incidence of false-negative readings.

In a further embodiment of a motility enrichment procedure, the competitor flagella antibodies are incorporated into the semisolid motility medium, without the use of a selective enrichment broth. Thus, the sample or an enriched sample is inoculated at the proximal end of the semisolid motility medium with competitor flagella antibodies and only the particular motile organism can travel to the distal end of the semisolid motility medium for detection.

In another aspect, the present invention provides a device for use in a competitor immobilization procedure to detect a particular motile organism, comprising a first compartment consisting essentially of a culture broth into which an enriched sample is added, a second compartment having a proximal end and a distal end, the second compartment communicating with the first compartment at the proximal end, and consisting essentially of a semisolid motility medium and

competitor flagella antibodies, and a means for detecting the particular motile organism, wherein the communication between the first and second compartments allows motile organisms to pass. It is not essential for the semisolid motility medium to contain selective agents because the competitor flagella antibodies confer selectivity.

Preferred means for detecting the particular motile organism include: a third compartment communicating with the distal end of the second compartment and consisting essentially of a medium that can visually change in the presence of the particular motile organism; and antibodies added to the distal end of the second chamber and diffusing proximally and essentially unidirectionally to produce an immobilization band upon interaction of the particular motile organism and the antibodies. A sample of distal semisolid motility medium is obtained after the particular motile organism has been incubated to allow movement to the distal end, for detection by pure culture methods. Other means for detecting the presence of the particular motile organism at the distal end of the second chamber include immunoassays and hybridization assays.

The competitor flagella antibodies are prepared using a mixture of bacterial strains of likely motile competitors. Preferably, as discussed herein, rabbits are immunized and the sera are pooled to obtain a mixture of competitor flagella antibodies.

Brief Description of the Drawing

The Figure illustrates an example of a competitor immobilization test device that incorporates competitor flagella antibodies into the semisolid motility medium, as a means for separating the particular motile organism from the motile competitors. The first compartment contains a broth into which a test sample or enriched sample is added. The first compart-

ment communicates with a second compartment that contains the semisolid motility medium with competitor flagella antibodies. The second compartment communicates with a third compartment that contains a means for detecting the particular motile organism. The first and third compartments do not directly communicate. The means for communication between the first and second compartments and the second and third compartments is by an opening, and preferably through a permeable barrier (e.g., frit) that allows movement of motile organisms but does not allow for mixing of the media between compartments.

Detailed Description of the Invention

As noted above, the present invention provides an improved process for detecting a particular motile organism using a competitor immobilization type assay, through the incorporation of competitor flagella antibodies within either or both of the culture media. This process reduces the incidence of false-negative results for detecting a particular motile organism, such as Salmonella, Escherichia coli, Listeria, or Yersinia entercolitica.

The preparation of competitor flagella antibodies is added into either or both of the selective enrichment medium and/or the semisolid motility medium at a concentration sufficient to retard the movement of the motile competitors. This concentration is dependent upon the titer of antibodies in sera. The titer varies from lot to lot and with the immunization program used.

The competitor flagella antibodies have multiple specificities to competitor motile organisms which may potentially be found in the sample. The competitor flagella antibodies are specific for the motile competitors and do not appreciably cross-react with the particular motile organism.

The motile organisms and motile competitors which can be found in food, water, waste water, environmental, pharmaceutical, cosmetic and clinical samples, include bacteria which are members of the genera Escherichia, Salmonella, Citrobacter, Enterobacter, Serratia, Hafnia, Proteus, Edwardsiella, Erwina, Vibrio, Listeria, Providencia, Bacillus, Pseudomonas, Aeromonas, Yersinia, Campylobacter, Cedecea, Morganella and Kluyvera. Species of Citrobacter include, for example, C. freundii, C. diversus and C. amalonaticus. Species of Salmonella include, for example, S. enterica, S. paratyphi-C, S. typhi, S. paratyphi-A, S. paratyphi-B, S. typhimurium, S. enteriditis, S. salamae, S. arizonae, S. diarizonae, S. houtenae, and S. bongori. Species of Serratia include, for example, S. liquefaciens, S. rubidaea, S. plymuthica, S. fonticola, S. odorifera, S. ficaria, S. grimesii and S. proteamaculans. Species of Escherichia include, for example, E. blattae, E. coli, E. adecarboxylata, E. hermannii, E. vulneris and E. fergusonii. Species of Erwinia include, for example, E. amylovora, E. ananas, E. cancerogena, E. carnegieana, E. carotovora, E. chrysanthemi, E. cypripedii, E. herbicola, E. mallotivora, E. milletiae, E. nigrifluens, E. paradisiaca, E. quercina, E. rhapontici, E. rubrifaciens, E. salicis, E. stewart, E. tracheiphila and E. uredovora. Species of Edwardsiella include, for example, E. hoshina, E. ictaluri and E. tarda. Species for Yersinia include, for example, Y. entercolitica, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. pseudotuberculosis and Y. ruckeri. Species of Enterobacter include, for example, E. amnigenus, E. cloacae, E. dissolvens, E. gergoviae, E. aerogenes, E. agglomerans, E. intermedium, E. nimupressuralis (NYV), E. sakazakii and E. taylorae. Species of Proteus include, for example, P. mirabilis, P. myxofaciens, P. vulgaris and P. penneri. Species of Morganella include, for example, M. morganii. Species

of Kluyvera, for example, K. ascorbata and K. cryocrescens. Species of Hafnia include, for example, Hafnia alvei. Species of Providencia include, for example, P. alcalifaciens, P. rettgeri, P. stuartii and P. rustigianii. In addition, motile organisms and motile competitors include species of Listeria, such as L. monocytogenes, L. invanovi, L. inocua, L. welshimeri, L. seeligen, L. grayi, L. murray, and L. jonesi.

Further strains of competitor organisms are disclosed in Ewing, "Edward's and Ewing's Identification of Enterobacteriaceae," 4th ed., Elsevier, New York, 1986, for Citrobacter freundii (pp. 350-62), Escherichia coli (pp. 116-19) and Serratia marcescens (pp. 431-40). The following examples of strains of Citrobacter freundii may be used to produce competitor flagella antibodies: Na 1a, Na 4, Md 10, Na 11, Mich 7, Mich 1, Md 2, Md 1, 5131/51, Mich 11, 3038, Br. Unit., and LC 54.

Examples of strains of Escherichia coli that can be used to produce competitor flagella antibodies include: Su 1242, Bi 7455-41, Bi 7327-41, U9-41, U4-41, A2-a, U5-41, AP 320c, Bi 7575-41, Bi 623-42, Su 4321-41, Bi 316-42, S 10018-41, E 39a, S 8316-41, P 12b, K 12a, A 18d, H 3306, U 11a-44, HW 23, HW 25, HW 26, HW 27, HW 28, HW 30, HW 31, HW 32, HW 33, HW 34, HW 35, BP 12665, 4370-53, 5017-53, P 11a, P 9b, E 3a, E 49, RVC 1787, P 9c, 149-51, 781-55, 4106-54, 5306-56, 1755-58, P4 and 2147-59.

Examples of Serratia marcescens that can be used to produce competitor flagella antibodies include CDC-863-57, CDC-836-57, CDC-862-57, CDC-864-57, CDC-866-57, CDC-680-57, CDC-841-57, CDC-877-57, CDC-1783-57, CDC-2420-57, CDC-827-57, CDC-874-57, CDC-1996-71, S-326(Traub), IP-389, Schweiz (Traub), IP-421, IP-250, IP-416, Sli (Traub), IP-987, IP-1114, IP-1041, IP-361, IP-2384 and IP-6672.

Competitor flagella antibodies are incorporated into either or both of the selective enrichment medium and/or the semisolid motility medium at a concentration high enough to prevent or substantially retard the movement of the motile competitors. The competitor flagella antibody preparation should not include any antibodies which appreciably react with and subsequently retard the movement of the particular motile organism to be detected. When the competitor flagella antibodies are added to the selective enrichment medium of a competitor immobilization test, the competitor flagella antibodies react with the flagella of the motile competitors and result in a clumping or agglutination of the motile competitor cells. When the competitor flagella antibodies are added to the semisolid motility medium, the competitor flagella antibodies restrict the ability of the motile competitors to move into and through the pores of the semisolid motility medium. Preferably, the competitor flagella antibodies are added to the semisolid motility medium to act as a means for separating the particular motile organism from the motile competitors.

Competitor flagella antibodies are prepared, for example, in rabbits using whole cell antigens (see, Edwards and Ewing, supra at pages 239-242) or purified flagella antigens of the motile competitors, using procedures such as described in Fey, Zbl. Bakt. Hyg., I., Abt. Orig A. 245:55-66 (1979), and Ibraham et al. J. Clin. Micro. 22:1040-44 (1985).

Other methods known to produce antibodies, including monoclonal antibody production techniques, may be used to produce the competitor flagella antibodies. Mixtures of monoclonal antibodies and/or polyclonal antibodies may form the proper recipe for the competitor flagella antibodies.

The competitor flagella antibodies are often a mixture of antibodies directed to epitopes on flagella

of the likely motile competitors in a particular sample. The competitor flagella antibodies can be a pool of antibodies specific for selected motile competitors or be a mixture of antibodies produced using a mixture of motile competitors. The titer of competitor flagella antibodies is determined by measuring motility inhibition of motile competitors with different concentrations of the competitor flagella antibodies. Preferably, the concentration of competitor flagella antibodies added to the selective enrichment medium and/or semisolid motility medium is selected to correspond to the lowest antibody concentration that blocks motility of the motile competitors in a test culture. An antibody titer of competitor flagella antibodies can be expected to be 1:500 to 1:15,000 using the motility inhibition assay described herein, depending upon the immunization procedure and specificity of the resulting antibodies.

Similarly, the effective concentration of competitor flagella antibodies should not inhibit the motility of the particular motile organism. This can be determined by testing multiple cultures of the particular motile organism in a motility-type test using specific concentrations of competitor flagella antibodies within the semisolid motility medium or the selective enrichment medium, or both.

Within one aspect, the competitor flagella antibodies are added to the selective enrichment medium and the semisolid motility medium of a competitor immobilization test. The sample or an enriched sample is inoculated into the selective enrichment medium. This results in the reaction of the motile competitors with the competitor flagella antibodies. This reaction could occur in the liquid selective enrichment medium and/or the semisolid motility medium. Preferably, the assay does not have a selective enrichment media, so the competitor flagella antibodies confer selectivity for the particular motile organism only in the semisolid

motility medium. Most preferably, the semisolid motility medium need not contain a selective agent to regulate movement of the motile organisms, because the competitor flagella antibodies function as a means for selecting for the particular motile organism over the motile competitors.

The reaction results in the inhibition of the movement of the motile competitors. For the competitor immobilization procedure, the competitor flagella antibodies act to restrict the movement of the motile competitors, while permitting the movement of the particular motile organism into and through the semisolid motility medium. If an immuno-immobilization detection technique is utilized, the antibodies specific to the particular motile organism are layered onto the distal surface of the semisolid motility medium as a means for detecting the particular motile organism, an immobilization band in the semisolid motility medium will be visible if particular motile organisms are present. Other detection techniques require the particular motile organism to reach the distal end of the semisolid motility medium.

The incorporation of the competitor flagella antibodies into the semisolid motility medium and/or selective enrichment medium acts as a method for conferring selectivity to a microbiological test, such as the BioControl 1-2 TEST®. In this way, the incorporation of the competitor flagella antibodies act to permit the unimpeded movement of the particular motile organism, resulting in the separation from the motile competitors and the "enrichment" of the particular motile organism. The incorporation of the competitor flagella antibodies into a semisolid motility medium permits the selection of the particular motile organism without the addition of selective agents, thus permitting motile organism strains, that might be inhibited by the selective agent, to grow to detectable levels. The incorporation of the

competitor flagella antibodies provides a method for conferring selectivity to a microbiological procedure without the use of selective agents.

The semisolid motility medium needs to have pores large enough to allow free movement of the motile organisms and have the necessary nutrients to allow nonselective growth of all motile organisms. The semisolid motility medium cannot approach the liquid state because there cannot be any mixing within the semisolid motility medium. Preferably, the semisolid motility medium should be an agarose preparation. The appropriate agarose concentration in a semisolid motility medium used in a motility immobilization procedure is influenced by the type of agarose employed.

In general, concentrations of agarose appropriate for use in a semisolid motility medium are in the range from about 0.1% (w/v) to about 0.5% (w/v).

The semisolid motility medium typically comprises agarose, hydrolyzed protein and other agents to assist the growth of the particular motile organism, such as vitamins. Preferably the hydrolyzed protein is a peptone such as the Difco peptones Bacto-peptone, Bacto-tryptone, Bacto-tryptose and Bacto-casitone. Concentrations of peptone range from about 1% (w/v) to about 5% (w/v). The use of other growth promoting agents include, for example, NaCl for Yersinia. It is preferred that sugars not be included in the semisolid motility medium because sugar metabolism often forms gas which can disrupt the semisolid motility medium.

An example of a suitable semisolid motility medium is 1.5% (w/v) polypeptone (BBL, Cockeysville, MD) and 0.2-0.3% (w/v) agar. The medium is cooled to 40-48°C prior to adding to its rigid support. The semisolid motility medium is allowed to solidify in its rigid support chamber.

The pore sizes of gels vary as a function of the concentration of agarose used and the type of

agarose employed. See Serwer et al., "Agarose Gel Electrophoresis of Bacteriophages and Related Particles III Dependence of Gel Sieving on the Agarose Preparation," Electrophoresis 4:232-36, 1983; and Serwer et al., "Exclusion of Spheres by Agarose Gels During Agarose Gel Electrophoresis: Dependence on the Sphere's Radius and the Gel's Concentration," Anal. Biochem. 158:72-78, 1986. The appropriate agarose concentration in the semisolid motility medium is empirically determined by the lowest concentration that permits rapid movement of the motile organisms but has sufficient mechanical strength to resist breakage when housed in a rigid support. Examples of a rigid support are a test tube or the motility immobilization device in the BioControl 1-2 TEST®. Examples of appropriate concentrations of various types of agarose preparations manufactured by Marine Colloids, Rockland, Maine, include (w/v): from about 0.1% to about 0.2% for HGT, from about 0.1% to about 0.2% for LE, from about 0.2% to about 0.25% for RE, and from about 0.25% to about 0.3% for Sea Plaque.

An enrichment method incorporating competitor flagella antibodies into a semisolid motility medium can be used to increase the levels of the particular motile organism relative to motile competitors for the benefit of detecting the particular motile organism using a detection assay. Examples of detection assays include, for example, a pure culture method, where differential agar plates are streaked and incubated to produce typical colonies of the particular motile organism; an immmunoassay procedure; a hybridization procedure; a latex agglutination procedure; an agglutination procedure and an immuno-immobilization procedure. An enrichment method of this type could be conducted, for example, by allowing a small sample (e.g., 0.1 gram of a fecal sample) or a small volume of a pre-enrichment medium (e.g., 0.1 ml) to contact the semisolid motility

medium containing the competitor flagella antibodies. The inoculated semisolid motility medium would need an incubation time from only about 2 to about 24 hours at a permissive temperature (usually 30°C to 37°C for most motile organisms, but sometimes 22°C to 27°C for certain motile bacteria, such as Yersinia entercolitica) or Listeria monocytogenes. Any visual indication of growth in the semisolid motility medium (the semisolid motility medium would become turbid or cloudy) would indicate presumptively that the particular motile organism is present. This might be considered as a presumptive positive result for the particular motile organism. More definitive determinations require a detection procedure either at the distal end of the semisolid motility medium or at a container communicating with the distal end of the semisolid motility medium, or by obtaining a sample from the distal end of the semisolid motility medium.

An inoculum obtained from the semisolid motility medium at a point distal from the original inoculation point could be taken and streaked onto differential agar plates to obtain a pure culture to confirm for the presence of the particular motile organism. Alternatively, the inoculum of the semisolid motility medium could be directly tested or introduced into a broth culture medium for a brief incubation time at a permissive temperature before conducting an immunoassay or a hybridization procedure.

The competitor immobilization procedure may utilize a device comprising a first compartment consisting essentially of a sample or an enriched sample and a broth and a second compartment having a proximal and a distal end, communicating with the first compartment at the proximal end and consisting essentially of a semisolid motility medium and competitor flagella antibodies. The communication between the first and second compartment allows for

movement of motile organisms but not for mixing of the media. Preferably, a porous plastic frit forms the barrier between the first and second compartments. In another embodiment, a third compartment communicates with the distal end of the second compartment, and contains a detection system or a culture medium to grow the particular motile organism for later detection. For example, the third compartment may contain a medium that turns turbid or can visually change in the presence of the particular motile organism.

The following examples are for purposes of illustration and not for limitation.

EXAMPLE 1

This example illustrates a comparison of a microbiological assay conducted with a competitor flagella antibody and without a competitor flagella antibody. The competitor flagella antibody specifically reacts with the motile organism, E. coli 0157:H7. The competitor flagella antibody is added into both the semisolid motility medium and the tetrathionate selective enrichment broth of a BioControl 1-2 TEST® at a final concentration of 1:200 (v/v). The BioControl 1-2 TEST® is a test specific for Salmonella as the particular motile organism. A strain of Salmonella and a strain of the serotype E. coli 0157:H7 are grown overnight in BHI broth at 35°C. The particular motile organism and the motile competitor are grown separately. The Salmonella culture is decimally diluted. The E. coli cultures are added into sterile test tubes at concentrations of approximately 10^7 cells/ml. Salmonella are then added into each of the E. coli test tubes from each dilution so that the concentration of E. coli remains constant in each tube while the concentration of Salmonella is reduced in consecutive tubes until extinction is reached. One-tenth ml from each test tube is added into the enrichment broth of a

BioControl 1-2 TEST® unit according to the manufacturer's instructions. Particular antibodies specific to the flagella of Salmonella are added to the distal surface of the semisolid motility medium through an opening provided in the container according to the manufacturer's instructions. The 1-2 TEST® units are then incubated for 24 hours at 35°C and observed for the development of an immobilization band. The results of this test are shown in Table 1 below and indicate that the presence of competitor flagella antibodies increase the sensitivity of the 1-2 TEST® and reduced the incidence of false-negative results.

TABLE 1

No. E. coli (CFU/ml) added to 1-2 TEST	No. Salmonella (CFU/ml) added to 1-2 TEST	Presence of Immobilization Band Without Competitor Antibodies	Presence of Immobilization Band With Competitor Antibodies
10^7	10^7	+	+
10^7	10^6	+	+
10^7	10^5	-	+
10^7	10^4	-	+
10^7	10^3	-	+
10^7	10^2	-	+
10^7	10^1	-	+
10^7	10^0	-	-

+ = Immobilization reaction resulting in the development of a strong, clearly identifiable band.
- = Weak or nonexistent immobilization band.

EXAMPLE 2

This example illustrates the preparation of a competitor flagella antibody prepared from an active motile broth culture of a motile competitor. Each culture is grown in infusion broth at 37°C for 15 to 18 hours. The cultures are then diluted with an equal volume of physiological saline containing 0.6% formalin.

Rabbits are immunized by marginal ear injection using the formalinized whole cell cultures and with the following immunization schedule: the first dose (0.5 ml of formalinized culture), second dose (1.0 ml), third dose (2.0 ml), fourth and fifth (4.0 ml each). The intervals between injections are 4 to 5 days. The rabbits are bled 7 to 10 days following the final immunization to obtain the sera that comprise the competitor flagella antibodies.

EXAMPLE 3

This example illustrates the competitor flagella antibody titer determination using the competitor flagella antibody produced in Example 2. The rabbits' sera of Example 2 are pooled, diluted and mixed with approximately 5 ml of sterile, liquefied and tempered (45°C) semisolid agar medium (3 g peptone, 0.3 g agarose in 100 ml water) in a test tube. The antibody-supplemented medium is permitted to solidify. Motile competitor organisms are stabbed into the semisolid medium and incubated for 18 to 24 hours at 35°C. The tube supplemented with the lowest concentration of antibodies showing no motility when compared to a controlled tube with no antibody supplementation is defined as the working titer of the sera against that specific strain of competitor organism.

EXAMPLE 4

This example illustrates a type of test used to ensure that the competitor flagella antibody of Example 2 does not cross-react with the particular motile organism. The pooled sera are adjusted to the appropriate working titer and supplemented into 5 ml of semisolid medium, as described in Example 3. Strains of the particular motile organism are stabbed into the semisolid medium and incubated at 35°C for 18 to 24

hours. The strains of the particular motile organism should show little or no inhibition of motility in the antibody-supplemented medium when compared to a control containing a semisolid medium with no supplementation of competitor flagella antibodies. If motility inhibition is seen with the particular motile organism, then the sera is passively adsorbed with the strain of particular motile organism showing inhibition, to remove the cross-reacting factor, according to the method of Ewing (Id. at pp. 118-119).

EXAMPLE 5

This example illustrates the use of competitor flagella antibodies in a semisolid motility medium to enrich for Salmonella in a food sample. A device containing three compartments that are separated by a porous plastic frit (100 μm pore) that permits unrestricted movement of the motile bacterial between compartments is used. A first compartment contains approximately 1 ml of buffered peptone water broth. The first compartment communicates with a second compartment that contains the semisolid motility medium with competitor flagella antibodies. A third compartment communicates with the second compartment at a location distal to the communication point of the first and second compartments. The third compartment contains 1 ml of m-Broth. The device used in this example is illustrated in Figure 1.

A food sample that has been incubated in a buffered peptone water pre-enrichment broth for 24 hours at 35°C is used as the inoculum. One-tenth milliliter of the pre-enrichment broth is inoculated into the first compartment. The device is allowed to incubate for up to 24 hours at 35°C. After incubation, the third compartment is observed for turbidity, presumptively indicating that Salmonella is present in the sample. A small aliquot of the broth from the third compartment is

removed and used to conduct an enzyme immunoassay procedure according to the accepted method of the Association of Official Analytical Chemists.

EXAMPLE 6

This example illustrates the use of competitor flagella antibodies in a semisolid motility medium to enrich for and detect E. coli 0157:H7 in a clinical sample. A device described in Example 5 is used. The first and third compartments contain approximately 1 ml of a medium containing 1.0%(w/v) peptone, 0.3% meat extract, and 0.5% sodium chloride. The semisolid medium in the second compartment contains the competitor flagella antibodies in a medium with 1.0% (w/v) peptone, 0.3% meat extract, 0.5% sodium chloride and 0.25% HGT agarose (Marine Colloids). The competitor antibodies permit the movement of the E. coli 0157:H7 strain while inhibiting the movement of other competitor motile strains in the semisolid medium.

One tenth gram of a fecal sample is added to the first compartment. The device is then incubated at 37°C for 18-24 hours. Any growth appearing in the third compartment presumptively indicates the presence of the E. coli strain in the sample.

The confirmatory detection step is accomplished by adding antibodies to the 0157 surface antigen of E. coli 0157:H7 coated onto latex beads to the turbid third chamber. The antibodies are available from Oxoid Ltd. (U.K). The unit is incubated for 60 minutes at 37°C and then observed for aggregation of the latex beads. Aggregation of the latex beads confirms for the presence of E. coli 0157:H7.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration,

various modifications maybe made without deviating from the spirit and scope of the invention.

Claims

I Claim:

1. A method for detecting the presence of a particular motile organism within a sample, comprising:

inoculating a sample or an enriched sample, at a proximal site, wherein said sample or enriched sample presumptively contains a particular motile organism and one or a plurality of motile competitors;

incubating the sample or the enriched sample with a semisolid motility medium containing a plurality of competitor flagella antibodies, wherein the plurality of competitor flagella antibodies inhibit the movement of the motile competitors throughout the semisolid motility medium, thereby allowing the motile organisms whose flagella are not bound with antibodies to move distally in the semisolid motility medium; and

detecting for the presence of the particular motile organism at a distal site.

2. The method of claim 1 wherein the detecting for the particular motile organism is achieved by an assay selected from the group consisting of pure culture assays, immunoassays, hybridization assays, immuno-immobilization assays, and combinations thereof.

3. The method of claim 1, further comprising a selective enrichment medium communicating with the semisolid motility medium at a proximal site.

4. The method of claim 1 wherein the selective enrichment medium contains a chemoattractant to delay movement of the motile organisms into the semisolid motility medium.

5. The method of claim 1 wherein the semisolid motility medium contains from about 0.5% to about 5% (w/v) peptone, competitor flagella antibodies and a gelling agent.

6. The method of claim 5, further comprising the addition of a chemoattractant to the semisolid motility medium, wherein the motile organisms metabolize the chemoattractant to a lower level, thereby having the motile organisms whose flagella are not bound with antibodies to move.

7. The method of claim 5 wherein the gelling agent is agar or agarose.

8. The method of claim 1 wherein the detection of the particular motile organism step comprises the addition of particular antibodies at the distal site, said particular antibodies present in sufficient quantity to produce an immobilization band in a semisolid motility medium upon interaction of the particular motile organism with the particular antibodies.

9. The method of claim 1 wherein the particular motile organism is Salmonella, Escherichia coli, or Yersinia entercolitica.

10. The method of claim 1 wherein the particular motile organism is a species of Listeria.

11. The method of claim 1 wherein the plurality of competitor flagella antibodies are directed to bacterial strains selected from the group consisting of Salmonella, Citrobacter, Escherichia, Serratia, Enterobacter, Edwardsiella, Hafnia, Proteus, Providencia, Morganella, Erwina, Campylobacter, Listeria, Yersinia, and combinations thereof.

12. The method of claim 11 wherein the species of Salmonella are selected from the group consisting of S. enterica, S. paratyphi-C, S. typhi, S. paratyphi-A, S. paratyphi-B, S. typhimurium, S. enteriditis, S. salamae, S. arizonae, S. diarizonae, S. houtenae, and S. bongori species; of Citrobacter are selected from the group consisting of C. freundi, C. diversus, and C. amalonaticus; wherein the

species of Escherichia are selected from the group consisting of E. coli, E. blattae, E. adecarboxylata, E. hermannii, E. vulneris, E. fergusonii; wherein the species of Serratia are selected from the group consisting of S. marcescens, S. liquefaciens, S. rabidaea, S. plymuthica, S. fonticola, S. odorifera, S. ficaria, S. grimesii, and S. proteamaculans; wherein the species of Erwinia are selected from the group consisting of E. amylovora, E. ananas, E. cancerogena, E. carnegieana, E. carotovora, E. chrysanthemi, E. cypripedii, E. herbicola, E. mallotivora, E. milletiae, E. nigrifluens, E. paradisiaca, E. quercina, E. rhapontici, E. rubrifaciens, E. salicis, E. stewart, E. tracheiphila and E. uredovora; wherein the species of Edwardsiella are selected from the group consisting of E. hoshina, E. ictaluri and E. tarda; wherein the species of Yersinia are selected from the group consisting of Y. enterocolitica, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. pseudotuberculosis and Y. ruckeri; wherein the species of Enterobacter are selected from the group consisting of E. aerogenes, E. agglomerans, E. intermedium, E. nimupressuralis (NYV), E. sakazakii, E. amnigenus, E. cloacae, E. dissolvenus, E. gergoviae and E. taylorae; wherein the species of Proteus are selected from the group consisting of P. mirabilis, P. myxofaciens, P. vulgaris and P. penneri; wherein the species of Morganella consists of M. morganii; wherein the species of Kluyvera are selected from the group consisting of K. ascorbata and K. cryocrescens; wherein the species of Hafnia consists of Hafnia alvei; and wherein the species of Providencia are selected from the group consisting of P. alcalifaciens, P. rettgeri, P. stuartii and P. rustigianii.

13. The method of claim 1 wherein the plurality of competitor flagella antibodies are directed to a species of Listeria.

14. In a method for detecting the presence of a particular motile organism within a sample by:

inoculating a selective enrichment medium containing at least one chemotactic attractant with a sample or an

enriched sample containing the particular motile organism and one or more motile competitors, said chemotactic attractant causing motility inhibition of the particular motile organism and the motile competitors, said enrichment medium being selective for the growth of the particular motile organism relative to the growth of the motile competitors;

contacting the selective enrichment medium containing the motile organisms with a semisolid motility medium containing the chemotactic attractant in a concentration which is less than the initial chemotactic attractant in the selective enrichment medium;

incubating the sample in selective enrichment medium under conditions which permit the motile organisms to metabolize the chemotactic attractant to a lower level than was initially in the semisolid motility medium, thereby allowing the motile organisms to move into and through the semisolid motility medium, said semisolid motility medium further containing particular antibodies specific for the particular motile organism, said particular antibodies diffusing into the semisolid motility medium from a position distal to the position of the motile organisms and present in sufficient quantity to produce a persistent immobilization band upon interaction of the particular motile organism with the antibodies; and

observing the formation of the immobilization band:

wherein the improvement comprises adding a plurality of competitor flagella antibodies to the semisolid motility medium, or the selective enrichment medium, or the semisolid motility medium and the selective enrichment medium whereby the plurality of competitor flagella antibodies immobilize the motile competitors, and acts as a means for separating the particular motile organism from the motile competitors.

15. The method of claim 14 wherein the plurality of antibody types are directed to bacterial strains selected from the group consisting of Salmonella, Citrobacter, Escherichia, Serratia, Enterobacter, Edwardsiella, Hafnia, Proteus, Providencia, Morganella, Erwina, Campylobacter, Listeria, Yersinia, and combinations thereof.

16. The method of claim 15 wherein the species of Salmonella are selected from the group consisting of S. enterica, S. paratyphi-C, S. typhi, S. paratyphi-A, S. paratyphi-B, S. typhimurium, S. enteriditis, S. salamae, S. arizonae, S. diarizonae, S. houtenae, and S. bongori; species of Citrobacter are selected from the group consisting of C. freundi, C. diversus, and C. amalonaticus; wherein the species of Escherichia are selected from the group consisting of E. coli, E. blattae, E. adecarboxylata, E. hermannii, E. vulneris, E. fergusonii; wherein the species of Serratia are selected from the group consisting of S. marcescens, S. liquefaciens, S. rabidaea, S. plymuthica, S. fonticola, S. odorifera, S. ficaria, S. grimesii and S. proteamaculans; wherein the species of Erwinia are selected from the group consisting of E. amylovora, E. ananas, E. cancerogena, E. carnegieana, E. carotovora, E. chrysanthemi, E. cypripedii, E. herbicola, E. mallotivora, E. milletiae, E. nigrifluens, E. paradisiaca, E. quercina, E. rhapontici, E. rubrifaciens, E. salicis, E. stewart, E. tracheiphila and E. uredovora; wherein the species of Edwardsiella are selected from the group consisting of E. hoshina, E. ictaluri and E. tarda; wherein the species of Yersinia are selected from the group consisting of Y. enterocolitica, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. pseudotuberculosis and Y. ruckeri; wherein the species of Enterobacter are selected from the group consisting of E. aerogenes, E. agglomerans, E. intermedium, E. nimupressuralis (NYV), E. sakazakii, E. amnigenus, E. cloacae, E. dissolvenus, E. gergoviae, and E. taylorae; wherein the species of Proteus are selected from the group consisting of P. mirabilis, P. myxofaciens, P. vulgaris and P. penneri; wherein the species of Morganella consists of M. morganii; wherein the species of Kluyvera are selected from the group consisting of K. ascorbata and K. cryocrescens; wherein the species of Hafnia consists of Hafnia alvei; and wherein the species of Providencia are selected from the group consisting of P. alcalifaciens, P. rettgeri, P. stuartii and P. rustigianii.

17. A device for a competitor immuno-immobilization test to detect a particular motile organism, comprising:

a first compartment consisting essentially of a sample or an enriched sample and a broth;

a second compartment, having a proximal and a distal end, communicating with the first compartment at the proximal end, and consisting essentially of a semisolid motility medium and competitor flagella antibodies, wherein the communication between the first and second compartments allows for movement of motile organisms but not for mixing of the media; and

a means for detecting the particular motile organism at the distal end.

18. The device of claim 17, further comprising a third compartment communicating with the second compartment at the distal end, wherein the communication between the second and third compartments allows for movement of motile organisms but not for mixing of the media.

19. The device of claim 17 wherein the means for detecting the particular motile organisms comprises the addition of a particular antibody to the distal end of the second chamber, and wherein said particular antibody diffuses in a proximal direction and essentially unidirectionally to produce a visible immobilization band upon interaction with the particular motile organisms.

20. The device of claim 17 wherein the means for detecting the particular motile organism comprises a detection technique with a sample of semisolid motility medium obtained at the distal site, wherein the detection technique is selected from the group consisting of pure culture techniques, immunoassays, and hybridization procedures.

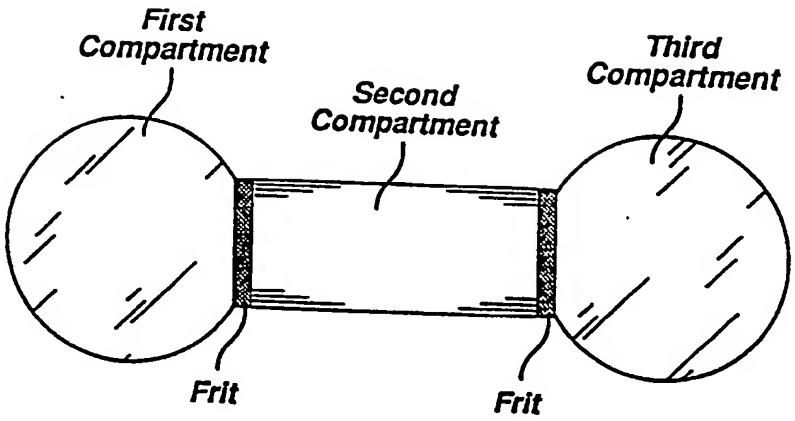
First Compartment
Second Compartment
Third Compartment
Frit
Frit

Figure 1

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/02022

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: G 01 N 33/569, C 12 M 1/34,//C 12 Q 1/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	G 01 N, C 12 Q

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	Biological Abstracts, vol. 67, no. 1, 1979, (Philadelphia, PA, US) B. Swaminathan et al.: "Rapid detection of Salmonellae in foods by membrane filter-disc immuno-immobilization technique", see page 235, column 2 and page 236, column 1, abstract 2410, & J. Food Sci, 43(5): 1444-1447,1978 --	1-9
A	EP, A, 0214340 (BIOCONTROL SYSTEMS, INC.) 18 March 1987 see the whole document --	1-9,17-20
A	GB, A, 2192185 (GENETIC SYSTEMS CORP.) 6 January 1988 see claims 1,2,6,7	1

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23rd July 1990	23 AUG 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9002022 SA 36420

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 20/08/90
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0214340	18-03-87	US-A-	4920063	24-04-90
		CA-A-	1271707	17-07-90
		JP-A-	62061598	18-03-87
GB-A- 2192185	06-01-88	US-A-	4834976	30-05-89
		AU-A-	7495887	07-01-88
		BE-A-	1000743	28-03-89
		DE-A-	3722098	03-03-88
		FR-A-	2601458	15-01-88
		LU-A-	86938	02-02-88
		NL-A-	8701554	01-02-88
		SE-A-	8702734	04-01-88
		JP-A-	63102697	07-05-88